SEASONAL CHANGES IN GIBBERELLIN-LIKE COMPOUNDS IN OVARIES AND YOUNG FRUITS OF NAVEL ORANGE

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENT	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION	1
LITERATURE REVIEW	3
General	3
Cultivar Selection, Cultural Practices and Environment and Their Effects on Navel Orange Fruit Set	5
Growth Regulators	7
Methods of Gibberellin Assay	15
METHODS AND MATERIALS	18
Analytical Method	18
Studies of Endogenous Gibberellins in Navel Orange Fruits	23
RESULTS	27
Analytical Method	27
Studies of Endogenous Gibberellins in Navel Orange Fruits	43
DISCUSSION	63
Analytical Methods	63
Studies of Endogenous Gibberellins in Navel Orange Fruits	66
SUMMARY	73
LITERATURE CITED	74
BIOGRAPHICAL SKETCH	95

LIST OF TABLES

Table		Page
1.	RFI of GA and GA_1 after reaction with sulfuric acid under different reaction conditions.	29
2.	Comparison of solvent flow, GA movement and GA definition on florisil, avicel and Eastman silica gel thin-layer plates.	31
3.	Comparison of fresh weights and frozen weights of navel orange fruit samples	36
4.	Comparison of RFI of sample and GA_1 solutions to RFI of propylated sample and GA_1 solutions separated by gas chromatography.	42
5.	RFI of processed sample portions from young fruit samples of 9 navel orange trees.	44
6.	RFI of samples collected for comparison of sample collection and storage methods and for determination of intratree variability.	45
7.	Gibberellin concentration and total amounts of gibberellins per fruit in samples from 4 navel orange trees during the early fruit growth period, 1967	47
8.	Average fresh weight and percent weight growth of ovaries and young fruits in samples collected for determinations of endogenous gibberellins during growth of the young fruits	50
9.	Average volume and percent volume growth of ovaries and young fruits in samples collected for determinations of endogenous gibberellins during growth of the young fruit	51
10.	Young fruit drop from the 4 navel orange trees used in the experiment for determining changes in endogenous gibberellin amounts	62

LIST OF FIGURES

Figure		Page
1.	Changes in RFI of decreasing concentrations of standard GA in 85% sulfuric acid. Measurements were made with an Aminco-Bowman spectrophotofluorometer, excitation maximum 410 mu, emission maximum 455 mu.	28
2.	RFI of processed 1-, 3-, and 10-gram portions of a single sample of navel orange fruits	34
3.	Mean RFI of processed samples to which GA was added. Each mean represents 3 determinations.	37
4.	Gas chromatogram of the diazopropane blank.	38
5.	Gas chromatogram of propylated GA	39
6.	Gas chromatogram of propylated GA_1.	40
7.	Gas chromatogram of a propylated fruit sample. Sample propylated after thin-layer chromatography but before treatment with sulfuric acid.	41
8.	Seasonal changes in gibberellin concentration and total amounts per fruit of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance	48
9.	Seasonal changes in average weight and volume of fruits of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance	52
10.	Seasonal changes in average weight and volume of fruits of samples of ovaries and young fruits of navel orange.	53
11.	Mean weekly percent growth rate of fruits of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance	54

12. Mean weekly percent growth in fruit weight and gibberellin concentration of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance. 56

13. Mean percent growth in fruit volume and gibberellin concentration of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance. 57

14. Scattergram of paired percent fruit weight growth and gibberellin concentration data of samples of ovaries and young fruits of navel orange 59

15. Seasonal changes in total gibberellins per fruit and mean weight of fruit of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance. 60

16. Seasonal changes in total gibberellins per fruit and mean volume of fruit of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance. 61

INTRODUCTION

The navel oranges constitute a group of very similar seedless cultivars of sweet oranges (Citrus sinensis Osbeck) that have long been known as shy or erratic bearers (290). Very few seeds are produced because of pollen sterility and defective embryo sacs, which results in unfruitfulness. Parthenocarpic production of fruit is commonplace but not sufficient for consistent, satisfactory cropping (75).

Nevertheless, the very high quality of navel oranges has stimulated their planting and testing in most citrus areas of the world. In California, where the 'Washington' navel orange is a major cultivar, navel oranges bear well in milder climatic areas but are subject to poor fruit set in warm, dry areas. In Florida, Arizona and Texas, navel oranges are grown only on a limited scale, due primarily to low yields caused by poor fruit set. Navel prices and market demand are strong and Florida growers would plant larger acreages of navels, if good fruit set and yields were assured.

Many attempts have been made to overcome poor fruiting of navel oranges. Research has shown the navel oranges to be more sensitive than seedy types to physiological stresses induced by unfavorable environmental conditions (44). Attempts have been made to improve yields through cross pollination (285), cultural practices (44), budwood selection (231), girdling (132), and applications of growth regulators (108, 133). These efforts have met with only limited success and the problem is still largely unsolved.

Gibberellic acid (GA_3, hereinafter referred to as GA) is the only growth regulator that has increased fruit set of the navel orange. GA applied to individual flowers and flowering shoots has effectively increased fruit set but has been ineffective when applied to entire trees (108, 133).

Only very limited research has been done on the kinds and amounts of gibberellin-like substances which are found in the navel orange tree (129, 257). More research is needed to develop an accurate, but simple, method for measuring these substances in citrus tissue, to determine ways in which amounts and kinds of gibberellin-like substances change within the plant as related to fruit growth, and to trace the absorption and translocation of gibberellins that are applied to the tree.

This study was designed to adapt a fluorometric method of analysis to the measurement of naturally occurring gibberellin-like substances (hereinafter referred to as gibberellins) in young navel orange fruit, to determine the native amounts of gibberellins in young fruit during the drop period, and to relate the amounts of gibberellins to young fruit growth.

LITERATURE REVIEW

General

The navel orange, as represented by its selected strains, is considered by many the standard of excellence of citrus fruits. Since its discovery about 1820 near Bahia, Brazil (40, 42, 206, 227), it has been introduced into most citrus areas of the world.

As 'Bahia' navel orange it was planted in Australia, New Zealand and South Africa (18, 42, 286). Several years later it was introduced into the United States from Australia, England and directly from Brazil (40, 42, 64, 116, 202, 203, 227, 245, 290). Most of the early importations were trees budded from bud mutants of poor fruit quality. In 1870, an extremely high fruit quality, good yielding strain was introduced from Brazil. As 'Washington' navel orange it received wide acceptance in California and was soon planted in Florida, Texas and Arizona.

From the United States, the 'Washington' navel orange was introduced into most of the world's citrus producing areas (76, 79, 111, 112, 114, 194, 216, 259, 260, 262, 289).

Since its discovery in Brazil, the navel orange has been recognized as an exceptional orange type, not only for its quality, but also for its shy and erratic bearing. Poor fruiting behavior has been reported from most citrus producing areas (18, 25, 42, 111, 112, 170, 261, 269, 270, 286).

Within the United States, the navel oranges were reported to be unfruitful in Arizona, the desert areas of California, and in Texas (64,

73, 74, 205, 264, 265, 287, 290). In Florida, where the introduction of the 'Parsons' navel orange preceded the introduction of 'Washington' navel, both varieties were noted as unfruitful even before the turn of the century (285).

Due to the high quality of the navel oranges, much work has been done to determine the cause of unfruitfulness of the navel orange. Webber (285), working in Florida with both 'Washington' and 'Parsons' navel strains, showed that these cultivars produced parthenocarpic fruit regardless of whether the flowers were emasculated or allowed to self-pollinate. Cross-pollination served only to slightly increase the seediness of the fruit. Osawa (187) and other Japanese workers determined the nature of navel orange parthenocarpy. Osawa observed that: parthenocarpic varieties of citrus do not require the stimulus of pollination to set fruit; disintegration of the pollen-mother-cells in 'Washington' navel orange occurs as early as the sporangeous stage and a great majority of them degenerated and disappeared in the stage of resting nuclei; no pollen grains were found in the anthers of 'Washington' navel orange in its flowering period; seedless fruits of 'Washington' navel orange were produced chiefly owing to the lack of fertility of pollen-grains and partly to the frequent disintegration of the embryo sacs; disintegration of the embryo sacs occurred primarily by breakdown of the megaspores which form the embryo sac. Later work in the United States (41, 288) bore out these findings.

Attempts have been made to overcome the poor fruiting tendency of navel oranges by selection of varieties with greater inherent fruit-setting ability and by the use of cultural practices which overcome environmental factors reducing fruit set. Since the discovery of auxins and other growth hormones, these materials have been tested to determine

their effect on fruit set of navel orange. Results of this research will be reviewed in the sections that follow.

Cultivar Selection, Cultural Practices and Environment and Their Effects on Navel Orange Fruit Set

Cultivar Selection

Within a few years after the original 'Washington' navel orange introduction, the high incidence of bud mutation in navel oranges was noted and several selected strains such as 'Thomson', 'Golden Nuggett', 'Golden Buckeye' and 'Navelencia' were recognized and described (45, 46, 159). The 'Thomson' navel was described as heavier yielding than the 'Washington'.

In 1911, Shamel and co-workers began an extensive program for selection of better quality, higher yielding strains of navel oranges (224, 225, 226, 228, 229, 230, 236, 237, 238). They concluded that: quantity of fruit is an inherent characteristic which can be maintained through bud propagation; and performance records indicate that superior yielding strains have been isolated through bud selection. Since Shamel's work in California, several reportedly higher-yielding strains have been found.

In Texas, the promising 'Texas' navel (64, 271) proved to be an erratic bearer (3, 74, 156, 157, 271). However, several new strains now show promise (157).

In Florida, very little selection work has been done with navel oranges. A number of strains are recognized but none has attracted general interest (81, 116, 290, 300). 'Parsons' navel, the earliest named strain, was known in Florida before the 'Washington' navel. The 'Parsons' and the 'Summerfield', first propagated commercially in 1930,

originated from an 1835 'Bahia' navel importation. The 'Dream', 'Glen Improved' and 'Surprise' navel strains are bud mutations from 'Washington' navel. 'Summerfield' and 'Dream' navels are the most widely planted at the present time in Florida. Several strains selected by the United States Department of Agriculture are now being evaluated (81). The Florida State Department of Agriculture has also been selecting navel strains since 1952 as part of the Citrus Budwood Program (178).

Cultural Practices and Environment

The effect of environmental conditions and cultural practices on fruit set and retention has been investigated by many workers. Coit and Hodgson (43, 44) concluded that the citrus plant, due to its origin in the tropics, was subject to high rates of water loss from its aerial portions. Two factors, high evaporation from relatively unprotected upper leaf surfaces, and high transpirational water losses through permanently open stomates in the lower leaf surfaces, contributed to this high rate of water loss.

In California, moisture stress is the apparent cause of most young fruit drop (15, 16, 43, 44, 69, 77, 93, 97, 110, 115, 120). It has been shown (43, 44, 110) that the leaves under moisture stress are capable of removing water from the fruit, reducing fruit growth and causing abscission. Also, the difference in rate of drop between smaller and larger fruit has been attributed to differences in water loss from the fruits themselves (93). The importance of a continual, adequate water supply throughout the year for good fruit set and growth has been shown (15, 120, 130, 217). Important to understanding of the complexity of water balance was the observation of the effects of high temperatures on reduction of water uptake (16).

Moisture relations within the plant are not the single controlling

factor for fruit drop of navel fruits. Several experiments (15, 77, 97, 115, 193) have shown a correlation between low fruit set and abnormally high temperatures and between low fruit set and combined moisture stress and high temperatures.

The apparent interrelationship between water availability, temperature and fruit set has been explained as an effect of water availability and temperature on the nutritional balance of the tree (9, 69, 77). In citrus, unlike many other plants, limitation of any factor in the internal nutritional balance of the tree causes fruit abscission rather than fruit size reduction. Even the competition of the new spring growth flush for stored food reserves has been proposed as the cause of early blossom drop (94).

Good site selection and management practices which maintain uniform, vigorous tree and fruit growth have reduced the severity of the fruit drop problem. Still, navel orange trees, even under the best management, do not yield as well as other orange varieties.

Girdling, known to increase fruit set in other plants (99, 113, 119, 168, 169, 282), has given inconsistent results on 'Clementine' mandarin (186) but has greatly increased yields of the 'Orlando' tangelo (132). Girdling of 'Washington' and 'Dream' navel oranges has given highly variable results and has not been a recommended practice (7, 8, 61, 152, 153, 232, 233, 234, 235). Krezdorn (132) suggests that girdling might be effective in promoting fruit set of young, bearing-age navel orange trees.

Growth Regulators

The Stimulus for Parthenocarpic Fruit Growth

Pollination is neither necessary nor advantageous for fruit set in

the navel orange (285, 288). Fruit set in the navel oranges and other citrus cultivars such as satsuma mandarins, 'Marsh' grapefruit, 'Eureka' lemon, 'Tahiti' lime and 'Jaffa' orange occurs by vegetative parthenocarpy (75, 185) and stimuli for fruit set, fruit growth and abscission prevention must come from sources other than the pollen or developing ovules (187, 288). Vegetative parthenocarpy represents the ultimate stage of independence of fruit development from seed development (5, 91, 136, 145, 173).

Once fruit set has occurred, however, ovaries of parthenocarpic fruits develop in the same manner as non-parthenocarpic fruits of the same variety or species (47, 52, 53, 87, 103, 277). The nature of the stimuli that control fruit growth and prevent fruit abscission is still largely undetermined whether fruit set occurs parthenocarpically or non-parthenocarpically. However, work with growth regulating substances has supplied evidence that fruit set and fruit growth are controlled by specific growth-regulatory substances of a hormonal nature (173, 247, 267).

Auxins and Fruit Set

Auxins have induced parthenocarpic fruit growth in both seedy and normally parthenocarpic fruit plants (52, 53, 56, 63, 80, 87, 136, 147, 176, 207, 277, 284, 296). It has been hypothesized that auxin produced in the seeds controls fruit set and that parthenocarpic fruit set is due to higher auxin content of the ovaries of parthenocarpic cultivars (88, 89, 149, 150, 165, 177). This idea was refuted by research which showed that the auxin content of the seeds was correlated with seed development but not with fruit growth (6, 47, 54, 92, 143, 144, 145, 146, 148, 172, 174, 215, 246, 297) and by the fact that many auxin-induced parthenocarpic fruits did not develop normally, indicating that

other growth-promoting substances were needed to promote normal fruit growth (188, 207, 250).

Convincing evidence that growth promoters other than auxin were necessary for fruit set and fruit growth was presented when it was found that gibberellic acid alone could set fruit and stimulate continued fruit growth (see later sections). This evidence has led to the conclusion that a complex inter-relation exists between the seed, the developing ovary tissues and chemical stimuli in the ovary and developing fruit, and that auxins and other compounds are involved (51, 78, 147, 190).

Gibberellins and Fruit Set

Although originally isolated from fungal sources, gibberellins are an essential part of the hormonal system regulating higher plant growth (2, 198, 253, 291). Gibberellins and gibberellin-like substances have been found in many higher plants (29, 60, 67, 104, 121, 122, 123, 128, 129, 164, 181, 195, 200, 209, 210, 211, 241, 257, 291) and particularly in seeds (2, 51, 190, 198, 199, 255, 278).

The stem apex and apical leaves (24, 123, 139, 140, 272, 273), roots (28, 29, 123, 195), floral parts (47, 201) and developing seeds (11, 48, 121, 182, 295) are sites of gibberellin synthesis and evidence has been presented that gibberellins move in the plant in a non-polar manner (31, 125, 135, 158, 278, 279, 280, 281, 301).

There is considerable evidence that plants possess an autoregulatory system for controlling synthesis and interconversions of gibberellins within the plant, that gibberellin synthesis, interconversion, binding and degradation are related to changes in physiological processes within the plant, and that different gibberellins elicit different plant responses (72, 84, 100, 101, 102, 105, 121, 142, 164, 167, 183, 184,

191, 254, 283). The more acidic gibberellins are considered the most active in promoting plant growth while neutral gibberellins have been considered as inactive, reserve forms (84, 102, 105, 273, 283).

Gibberellins have been shown to influence many physiological processes of plants. This review will be limited to those influencing fruit set and fruit growth.

That gibberellins can promote and maintain growth has been well established. Gibberellins have been shown to stimulate growth by cell division in some plants and by cell enlargement in others (14, 19, 24, 95, 141, 158, 219, 220, 279), and to increase the rate of photosynthesis (4, 106, 163) and mobilization of metabolites to actively growing plant parts (171, 212, 213). Although there is still considerable difference of opinion on this point, it has been suggested that gibberellin stimulates growth by interaction with auxin (127, 134, 175, 196, 222, 223) and auxin has been shown to stimulate movement of carbohydrates and minerals to areas of growth (26, 131, 155, 160, 162, 256). Recent research has shown that gibberellin action may precede auxin action (179, 223).

Two different views regarding the effect of gibberellins on growth have been presented. Paleg (190) expresses the view that gibberellin acts in meristematic tissues of the plant through enhancement of auxin action and control of auxin levels. Gibberellin-induced growth could be the result of auxin increases in the same area in which gibberellin synthesis occurs, or the result of gibberellin movement from the site of synthesis, or application, to another site where physiological responses are stimulated by an increase in auxin production. Auxin produced in the fruit could stimulate fruit growth (87) and prevent fruit

abscission (1, 144). This theory has been supported by research showing a correlation between auxins and fruit growth (47, 52, 103, 143, 172).

Crane (51) proposes that pollination, fertilization, high growth promoter content in vegetative parthenocarpy, or application of growth regulators promote fruit set by establishment of a high rate of metabolic activity in the ovary. The resulting flow of metabolites and growth regulators from other parts of the plant maintains fruit growth and prevents abscission. This theory was strengthened by research which shows that developing embryos and fruit have a strong polarizing effect on the movement of metabolites to the fruit (96, 138, 258), that gibberellin stimulated parthenocarpic fruit growth where auxins did not (50, 56, 57), that seed-produced auxin was not necessary for fruit growth (208), and that auxin movement from the plant to the fruit rather than the reverse might control fruit abscission (83, 146, 298, 299).

Gibberellic acid has caused fruit set in plants which do not respond to auxin applications (35, 56, 57, 107, 133, 208, 244). In some plants, gibberellins caused fruit set as well or better than auxins (27, 55, 59, 61, 62, 86, 207, 208, 218, 221, 293, 295). Synergistic (66, 125, 208, 214) and antagonistic (49, 267, 268) effects between gibberellins and auxins in promoting fruit set have also been shown.

In summary, neither auxins nor gibberellins have been shown to individually control fruit set and fruit development in all plants. Gibberellins and auxins may, or may not have, distinct actions on growth systems, their action is not the same for all plant types nor for all varieties, species or genera within a plant group and their mode of action within a single plant type may not be the same when fruit set and fruit growth are controlled by endogenous growth regulators as when exogenous growth promoters are applied. Thus, each plant type must be

studied individually to determine the specific factors controlling fruit set and fruit growth.

Growth Regulator Effects on Citrus Fruit Set and Growth

Navel orange trees bloom heavily but less than 1% of the blossoms produce mature fruit (69). There is heavy drop of degenerative flowers and entire flowers but maximum shedding occurs in California when the young fruits are from 0.5 to 2 cm. in diameter (43, 44, 69). This drop is described as 'June' drop, since navel fruits reach this size during the month of June. In Florida, where climatic conditions are more variable during the blossoming and early fruit growth period, the time of full bloom, degree of flower abscission and degree of young fruit abscission are more dependent on environmental conditions and vary widely between growing seasons (30, 103). Blossom and young fruit drop occur during stage I (the stage of fruit growth when growth is largely through cell division) and during the early part of stage II (the stage of fruit growth when growth is largely through cell enlargement) (10, 68, 109). During stage II, the young fruits are considered very susceptible to factors affecting their rate of growth (43, 44, 154, 275, 276).

Since shedding of flowers and fruits is true abscission (44), most growth regulators found to influence abscission have been tested on citrus.

Auxins have stimulated parthenocarpic fruit set of citrus (87) and blossoms and young fruit of parthenocarpic citrus cultivars have been shown to contain higher amounts of auxins than blossoms of seedy cultivars (88, 89, 90, 103). In spite of conflicting evidence from plants other than citrus (90), higher amounts of auxins in ovaries of parthenocarpic citrus cultivars is still considered a causal factor of natural citrus parthenocarpy by several workers (88, 89, 103, 137).

Attempts to improve fruit set of 'Washington' navel orange and 'Marsh' grapefruit with naphthaleneacetic acid (204), 'Washington' navel and 'Valencia' oranges with 2,4-D (251) and 'Clementine' mandarin with 2,4-D (186) have been unsuccessful. Auxins have successfully reduced pre-harvest drop of 'Pineapple' orange in Florida (82, 240), 'Valencia' and 'Washington' navel oranges in California (248, 251, 252), and lemons in California (249).

Following the first reports of promotion of fruit set by gibberellins, these materials were tested on citrus. Increased fruit set was obtained in California when solutions of the potassium salt of GA (KGA) were brushed on individual flowers or sprayed on single branches of 'Washington' navel orange and 'Clementine' mandarin (35, 107, 243). However, KGA applications resulted in fruits of higher percent acid, reduced color, increased rind thickness and undesirable fruit shape.

When KGA was sprayed on entire trees, other problems developed. On 'Valencia' orange (39), slight increases in yields were accompanied by considerable leaf drop, thorn production and many adverse effects on the previous year's crop which was maturing at the time of the KGA applications (244).

When KGA was applied to entire trees of 'Clementine' mandarin, concentrations of 100 ppm to 500 ppm increased fruit set but tree damage and lower fruit quality resulted. Whole-tree sprays of KGA on grapefruit (37) caused severe leaf drop and delayed color loss from the fruit rind.

After testing KGA for 3 years on 'Washington' navel orange, Hield, et al. (108) concluded that KGA applied to entire trees decreased fruit set, caused serious twig dieback and leaf drop, increased rind thickness, and exerted a retarding effect on fruit color development. It was

hypothesized that commercial potential exists for KGA as a fruit set promoter only if its application could be limited to the flowers.

In California, the delaying effect of KGA on fruit color loss has been successfully used to lengthen the harvest period for lemons (32, 33, 36, 38). Also, in low concentrations, KGA has been used to reduce rind staining and other disorders of navel oranges (34).

In Florida, dipping of flowering shoots in gibberellin solutions has increased fruit set of 'Orlando' tangelo, 'Dream' navel orange, and 'Valencia' orange (133). Concentrations from 50 ppm to 1000 ppm were tested, with peak effectiveness at 500 ppm. There was a slight malformation and excessive splitting of fruits of 'Dream' navel and 'Valencia' oranges. In field tests of entire-tree sprays, GA increased fruit set of 'Orlando' tangelo but did not increase fruit set of 'Dream' navel and 'Valencia' trees. No vegetative responses were noted on either of these orange cultivars but thickening of fruit stems, increased leaf drop and increased fruit splitting were noted on 'Orlando' tangelo.

Additional unpublished work in Florida showed that 2.5 ppm and 5 ppm GA sprays at full bloom significantly increased 'Orlando' tangelo fruit set and caused no adverse vegetative effects.

Gibberellins and auxins are known to occur naturally in citrus tissue and both have promoted fruit set in several citrus cultivars. Thus, both gibberellins and auxins may be causal factors of natural citrus parthenocarpy. In order to clarify the specific effects and inter-relations of gibberellins and auxins in citrus fruit growth, considerable additional research on biochemical, histological and morphological changes occurring in young citrus fruits is needed.

Methods of Gibberellin Assay

Many methods have been used for quantitative and qualitative assay of gibberellins in higher plants. These have been discussed in several reviews (23, 161, 190, 198, 199, 294). Most assay of gibberellins and gibberellin-like substances has been done using bioassays. Physico-chemical methods have been used primarily for qualitative characterization.

Many difficulties are encountered when plants or plant parts are used to assay plant growth regulators. Plant responses to growth regulators are influenced by light, temperature and moisture conditions, and vary with the species used, the plant part used and the stage of development. Toxic substances, inhibitors and deactivation of the growth regulator during extraction and assay also affect bioassay results (161). Further, considerable time and laboratory space may be required to grow and manipulate the plant material. Physico-chemical methods overcome many of the limitations of bioassays but are generally not able to detect concentrations as low as those detected by bioassays. Also, plant extract and solvent impurities interfere in most physico-chemical assays and must be removed.

Bioassays for Gibberellins

Intact plants, plant parts, and seeds have been used in bioassays of gibberellins and gibberellin-like substances. Many intact-plant bioassays such as the rice seedling (166, 167, 181), Lunaria annua stalk elongation (23), cucumber hypocotyl (21, 22), dwarf pea (20) lettuce hypocotyl (71) and lettuce radicle (191) bioassays are based on the ability of gibberellins to overcome light-imposed growth reduction in the stems of higher plants.

Single-gene dwarf mutants, in which lack of certain genes prevents

biosynthesis of endogenous gibberellins, are used in the dwarf corn (197), dwarf bean (23) and Japanese morning glory (180) bioassays.

Gibberellin-stimulated growth of excised plant parts is measured in the dwarf pea epicotyl (195, 196) and maize leaf section (72) bioassays. The rice endosperm (181) and barley endosperm (189, 192) bioassays are based on gibberellin-stimulated production of alpha-amylase in germinating seeds. Gibberellin retardation of chlorophyll loss from senescing leaves is the basis for newly-developed bioassays using leaf discs of dandelion (70) and broad-leafed dock (292).

Physico-chemical Assays for Gibberellins

Although a highly sensitive, specific physico-chemical assay could be used advantageously in gibberellin research, the chemical nature of these compounds has made development of such as assay very difficult. Gibberellins are colorless, polycyclic, unsaturated compounds having no chemical characteristics which permit chemical assay at low concentrations (13, 126, 266).

A colorimetric test (239) has been used to detect amounts of GA in excess of 5 ug. Also, GA in concentrated sulfuric acid will yield a red color (58). Neither of these methods is sufficiently sensitive, however, to detect the low concentrations of gibberellins found in plant tissues.

Isotopic procedures have been attempted with unsatisfactory results. Carbon-labelled GA has been prepared and used in translocation experiments (301). Low activity and uncertainty regarding the purity of this biosynthetically produced material have seriously limited its use. Tritium-labelled GA has been made and used (135) on a very limited scale.

Gas chromatography has been a useful method for determining amounts

and kinds of gibberellins in plant extracts (117, 118). The need for complete removal of impurities in the samples and sensitivity of only 0.05 ug has restricted the use of this assay method. Infra-red spectra of gibberellins have also been used as a qualitative assay (257, 274).

Fluorescence assay has been the most successful non-bioassay method for the gibberellins. Kavanaugh and Kuzel (126) first demonstrated that GA, in concentrated sulfuric acid and excited by ultraviolet light, fluoresced characteristically. Fluorescence intensity increased linearly as GA concentration increased from 0.006 ug to 3.2 ug per ml. After it was found that plants contain a number of different gibberellins and that these could be separated by chromatographic techniques (2, 17, 67, 122, 124, 151, 291), the fluorescence characteristics of all gibberellins were determined (67). These fluorescence characteristics have been used for qualitative assay of many gibberellins and gibberellin-like substances.

Elson, et al. (67) first used fluorescence intensity as a quantitative assay for gibberellins. Gordon and Pankratz (85), using the basic technique of Kavanaugh and Kuzel (126) and improved thin-layer chromatographic techniques for removal of interfering substances, quantitatively assayed for GA in several plants.

GA_1 has been identified from citrus tissue (257) and GA has increased fruit set when applied to navel flowers (108, 133). Since these gibberellins have similar fluorescence characteristics (67, 263), relative fluorescence intensity appears suitable as a quantitative method in citrus.

METHODS AND MATERIALS

Analytical Method

The basic procedure of Gordon and Pankratz (85) was used. Since this method was developed for determination of GA in large samples of plant tissue, modifications were necessary for adapting the procedure to small samples of citrus fruit tissue and to estimate the effect of GA_1 on the quantitative procedure. GA_1 is the only gibberellin shown to be present in citrus tissue (257).

Suitability of Relative Fluorescence Intensity for Quantitative Analysis of Citrus Gibberellins

Sensitivity of the Aminco-Bowman spectrophotofluorometer to GA.--All determinations of relative fluorescence intensity (RFI) were made using the Aminco-Bowman spectrophotofluorometer. RFI of standard GA solutions was determined in order to establish the sensitivity of the spectrophotofluorometer to gibberellic acid. A standard GA solution was prepared by dissolving 2 mg of GA in 2 ml of ethyl acetate. From this standard, an aqueous solution containing 120 ug GA per ml was prepared. Dilutions were made of the aqueous solution to obtain final concentrations from 4.88 mug to 20 ug GA per ml. One ml of the aqueous stock solution for each GA concentration was placed in a 22 x 200 mm test tube and cooled for 5 minutes in an ice bath. For the control, one ml of distilled water was placed in the test tube. Five ml of cold 85% sulfuric acid was then added to each test tube and the contents thoroughly mixed. The test tubes remained in the ice bath for 10 minutes after mixing. After standing at room temperature for one hour, RFI of each GA concentration was determined on the spectrophotofluorometer.

Chromatography of GA and GA_1 on silica gel thin-layer plates.--Acidic extracts of plant tissue have been shown to contain both GA and GA_1 (67, 257). GA and GA_1 also move in a similar manner in most chromatographic systems (67, 122, 124). This similarity of movement was verified for the chromatographic system used in this research. After selection of the Eastman Chromagram thin-layer plate as the most suitable (see Results section), standard ethyl acetate solutions of GA and GA_1 were spotted 2 cm from the base of a 20 cm x 20 cm Eastman plate and the plate was developed using the prescribed solvent system (chloroform: acetic acid: methanol, 75:24:3). Gibberellin was detected by spraying with 90% ethanolic sulfuric acid (ethanol: sulphuric acid, 90:10), heating at 100^{o} C for 5 minutes and viewing under long wave ultraviolet light (365 mu).

Comparative RFI of equal concentrations of GA and GA_1.--Fluorescence excitation and emission maxima of GA and GA_1 are identical (67, 263) but comparative RFI of equal concentrations of the two gibberellins has not been firmly established (67, 263). Elson, et al. (67) treated equal concentrations of GA and GA_1 with 50% ethanolic sulfuric acid and found that the resulting fluorescence intensity of GA_1 was only one-tenth of the intensity of GA. Theriault, et al. (263), however, treated equal concentrations of GA and GA_1 with concentrated sulfuric acid at several different temperatures and concluded that at 0^{o} C fluorescence of GA_1 in sulfuric acid was minimal (less than 10% of GA fluorescence intensity). At higher temperatures fluorescence intensity of GA_1 and GA were approximately equal. Due to the effects of differing temperature, sulfuric acid concentration and mixing conditions on RFI of gibberellins, comparative RFI of equal GA and GA_1 standards was determined under the conditions used in this research.

Four 1 ug aliquots of standard ethyl acetate GA solutions and four 1 ug aliquots of standard ethyl acetate GA_1 solutions were placed in individual test tubes and 2 ml of water was added to each tube. Ten ml of sulfuric acid was then added to each tube in 4 different ways. One tube containing GA_1 and another containing GA were placed in an ice bath, cold 85% sulfuric acid added and the tubes chilled for 10 minutes after reaction. Another pair of tubes was treated in the same way as the first pair except that the tubes were heated at 50° C for 25 minutes immediately after mixing of the gibberellins and sulfuric acid. The other 2 pairs of gibberellin solutions were reacted at room temperature with 50% ethanolic sulfuric acid (ethanol: sulfuric acid, 50:50). One pair was then placed in a 50° C water bath for 25 minutes and the other pair in a 100° C water bath for 25 minutes. The reacted solutions were allowed to stand overnight and RFI of the solutions was determined.

Modifications of the Quantitative Method

Selection of the most suitable thin-layer plate.--Silica gel thin-layer sheets have been shown to effectively separate GA and GA_1 from other gibberellins and interfering substances (67, 122, 124), and prepared silica gel thin-layers on polyester backing have been found quick and convenient to use. Eastman Chromagram plates type K301R2 were compared to avicel and florisil plates to determine if this type of material was suitable for thin-layer chromatography of GA and GA_1. Time and distance of GA movement in the 3 types of TLC were determined. Five ug portions of standard GA were spotted 2 cm from the base of 4 different types of chromatographic plates: florisil without prewash, florisil with prewash, avicel and the Eastman TLC plate. The 4 were developed under identical conditions using the prescribed solvent system until the solvent front was 15 cm from the origin. The plates were dried

for 5 minutes, sprayed with 90% ethanolic sulfuric acid, heated until dry and observed under ultraviolet light.

Other modifications of the quantitative method.--Young fruit samples were collected, extracted, chromatographed and their RFI determined. Modifications were made in the procedure until GA could be consistently measured from aliquots representing 1 gram fresh weight of young fruit tissue.

Variability of the quantitative method.--Variability of the total quantitative procedure was estimated. A single sample was ground and extracted with acetone. After filtration of the ground sample, removal of the acetone and filtration of the aqueous solution, 4 equal portions of the sample were quantitatively processed and the 4 results compared.

Influence of sample size on RFI.--In all preliminary work to modify the analytical method, 1-gram portions of the sample were used. Improvements in sampling methods permitted collection and use of 3-gram samples. A single sample was ground and extracted. After removal of the acetone and filtration, comparison was made of RFI for 1-, 3-, and 10-gram sample portions to determine if higher amounts of interfering substances in the larger samples might dampen the RFI readings.

Reliability of the sample weighing procedure.--Since moisture condensation on the surface of the frozen samples was a possible source of error, the reliability of the weighing method was investigated. Three young navel trees in containers were transferred to the laboratory. A sample, consisting of all flower ovaries on the tree, was taken from each tree. The three samples were weighed at the time of collection, frozen and weighed again 6 hours later. Deviation of the frozen weights from the fresh weights was calculated.

Reliability and Sensitivity of the Fluorometric Procedure

After all modifications had been incorporated into the quantitative procedure a single large sample was ground and extracted and identical 1-gram fresh weight portions of the aqueous sample solution were used as background for a triplicated sensitivity and variability test. Each of 5 different amounts of standard GA (0.03125, 0.0625, 0.125, 0.25, and 0.5 ug) were added to 3 sample portions and the samples processed using the modified quantitative procedure. Since the final volume of the samples was 12 ml, the final concentrations of added GA were 0.0026, 0.0052, 0.0104, 0.0208 and 0.0417 ug per ml.

Verification of Gibberellin Activity in Processed Samples

That gibberellin-like substances are present in acidic, ethyl acetate-soluble fractions of plant tissue, extracted by methods similar to those used by Gordon and Pankratz, has been shown (12, 263). Since several modifications were made of this procedure, additional verification of the gibberellin-like nature of the samples was made. Gas chromatographic comparison of propylated samples to propylated GA and GA_1 standards, activity in the barley endosperm bioassay and fluorescence spectra were used.

Gas chromatographic characteristics.--For the gas chromatographic studies, several samples were processed to the point of final fluorescence development. Double distilled solvents were used to develop and elute the chromatograms in order to reduce the amount of interfering substances. Two samples were reacted with diazopropane for 1 hour, dried with nitrogen gas and redissolved in ethyl acetate. Propylated GA and GA_1 standards and blanks were made in the same manner. The samples, blanks and standards were injected into an F and M model 400 gas chromatograph equipped with 15% SE 30 on Chromosorb W column and

flame ionization detector. Injector, column and detector temperatures were 275° C, 250° C, and 280° C respectively. The gas chromatograms of the samples and the GA and GA_1 standards showed a number of similar characteristic peaks. To verify that the portion of the samples containing these characteristic peaks was also the portion responsible for the gibberellin fluorescence of the samples, the flame ionization detector was extinguished and this portion of several sample injections was trapped in a teflon tube immersed in a dry ice-acetone bath. The trapped portions of the sample were treated with 85% sulfuric acid and RFI determined and compared to sample aliquots which had not been passed through the column.

Barley endosperm bioassay.--For the barley endosperm bioassay, 2 samples were processed to the point of final fluorescence development and then assayed using the method of Paleg and co-workers (189, 192).

Fluorescence specta.--Fluorescence excitation and emission spectra of processed samples were compared to published data on fluorescence characteristics of the gibberellins (67) and to standard GA reacted with 85% sulfuric acid in the laboratory.

Studies of Endogenous Gibberellins in Navel Orange Fruits

Three experiments were conducted in this phase of the research. In the first, intertree variability of gibberellins in the fruit was determined. In the second, 3 methods of sample collection and storage were compared and an estimate of intratree sample variability was secured. The third experiment was the major experiment for determining changes in endogenous amounts of gibberellins in ovaries and young fruits during the fruit set and early fruit growth period. In addition, fruit drop counts were made to define the major young fruit drop period.

Sampling and Sample Handling Methods

To determine endogenous gibberellin amounts in ovaries and young fruits, samples were collected from 4 uniform trees in a commercial grove of 20-year old 'Washington' navel oranges near Citra, Florida. Samples for the other experiments were taken from other trees in the same grove. Total sample weight and numbers of fruit per sample varied according to the size of individual fruits at the time of sampling.

All samples consisted of axillary, singly-occurring ovaries or fruits of the leafy bloom and represented the average size and condition of similar fruits on the tree at the time of collection.

During collection, individual ovaries or fruits were frozen immediately by placing them in a collection container suspended in a dry-ice and acetone bath. The collected samples were held in the frozen state until processed in the laboratory.

Quantitative Analysis of Ovary and Young Fruit Samples

Each sample was removed from the freezer, poured into an insulated cup and weighed. Diameter of each ovary or fruit in the sample was measured and the average fruit weight and fruit diameter were calculated.

After weighing and measuring, the sample was quantitatively analyzed using the modified procedure described in the Results section.

After processing and fluorescence development, RFI of samples was compared to RFI of freshly prepared GA standards and blanks, and the amount of gibberellins was calculated.

Intertree Variability in Gibberellin Amounts

Thirty fruits from each of 9 trees were collected on April 28, 1966. Sample weight varied from 12.98 grams to 17.13 grams. After grinding, filtration and removal of the acetone from the filtrate of each sample, 2 portions were taken. One portion represented 1 gram

fresh weight of ovary tissue and the other 3 grams. The 18 sample portions were quantitatively analyzed, RFI determined and statistical analysis used to determine intertree variability.

Sample Collection and Storage Methods and Intratree Variability in Gibberellin Amounts

Six samples of 10 fruits per sample were taken from the same tree on April 25, 1967. Sample weight varied from 32.32 grams to 37.22 grams. Three methods of sample collection and handling were compared. In the first, samples were collected, frozen immediately, held frozen until arrival in the laboratory and then immediate quantitative analysis was performed. In the second method, the samples were collected, frozen immediately, held frozen for 3 weeks and then analyzed for gibberellin content. In the third method, the samples were collected, taken to the laboratory in the unfrozen condition where they were immediately analyzed for gibberellin content. Two samples were handled in each manner.

In the laboratory, each sample was ground, filtered and the acetone removed. Two equal portions of the aqueous portion of each sample were taken for quantitative analysis. The sample portions represented 3 grams fresh weight of ovary tissue. After RFI was determined, statistical analysis was performed to estimate variability of the laboratory procedure, variability between samples from the same tree and differences due to sample collection and storage method.

Changes in Endogenous Gibberellin Amounts

One sample was taken from each of 4 trees at weekly intervals from full bloom until young fruit drop was completed. Weekly samplings began on March 21, 1967, and continued until May 30, 1967. Samples consisted of 150 ovaries at the beginning of the sampling period and the number of fruits dropped progressively to 6 fruits in the final samples. Total

weight of the samples increased from 6 grams in the first samples to 180 grams in the final samples.

After grinding, filtration and removal of acetone from each sample, 1 portion of the filtrate was taken representing 3 grams fresh weight of ovary tissue. RFI was determined and statistical analysis used to compare gibberellin amounts and young fruit growth.

Estimation of the Major Young Fruit Drop Period

The major fruit drop period was estimated by counting fruit drop from full bloom until early drop ceased. Fruit drop estimates were obtained both from fruit collected from screens placed below the tree and from periodically counting fruits on tagged branches. Four large saran cloth screens were placed under the canopy of each of the 4 trees in the experiment. Each week, at the time of sample collection, all dropped fruit were counted and the screens cleaned and replaced. For the percent fruit drop data, 4 large branches were tagged on each of the 4 trees. A count of all flowers and fruit on the 4 branches of each tree was made. The number of flowers and fruit remaining on the 4 tagged branches of each tree was counted weekly and the number of flowers and fruits that had dropped during the week was calculated as a percentage of the original number at the beginning of the week.

RESULTS

Analytical Method

Suitability of RFI for Quantitative Analysis of Citrus Gibberellins

Sensitivity of the Aminco-Bowman spectrophotofluorometer to GA.--RFI of GA treated with 85% sulfuric acid at 0° C was determined for concentrations from 20 ug per ml to 4.88 mug per ml. RFI of the lower concentrations tested is shown in Fig. 1. The lowest concentration tested (4.88 mug per ml) was detected by the Aminco-Bowman spectrophotofluorometer and the concentration vs. RFI curve was approximately linear from 4.88 mug per ml to 312 mug per ml.

Chromatography of GA and GA_1 on silica gel thin layer plates.--Movement of GA and GA_1 on Eastman TLC plates using a solvent system of chloroform, acetic acid, methanol (75:24:3) was determined. GA and GA_1 chromatographed in this thin-layer system moved identically and the R_f of both materials was approximately 0.57.

Comparative RFI of equal concentrations of GA and GA_1.--RFI of GA and GA_1 solutions treated with sulphuric acid under different conditions is shown in Table 1. Although GA_1 fluorescence intensity was only 47% of the GA fluorescence intensity under the conditions used in the Gordon and Pankratz procedure (85% sulfuric acid at 0° C), GA_1 fluorescence was appreciable after treatment with sulfuric acid at 0° C. Therefore, changes in GA_1 concentration, as well as changes in GA concentration, were detectable when samples containing both gibberellins were treated with 85% sulfuric acid at 0° C, the procedure used in this research.

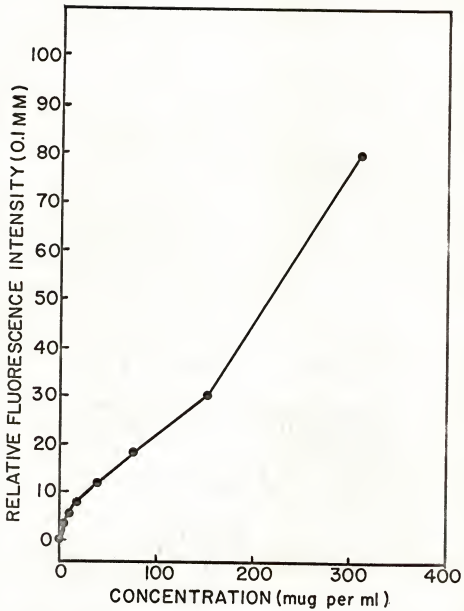

Fig. 1.--Changes in RFI of decreasing concentrations of standard GA in 85% sulfuric acid. Measurements were made with an Aminco-Bowman spectrophotofluorometer, excitation maximum 410 mu, emission maximum 455 mu.

Table 1.--RFI of GA and GA_1 after reaction with sulfuric acid under different reaction conditions.

	Relative fluorescence intensity[1]			
	85% H_2SO_4		50% H_2SO_4	
Sample	0° C no heating	0° C plus 25° C for 25 minutes	Room temperature plus 50° C for 25 minutes	Room temperature plus 100° C for 25 minutes
GA	17	17	13	13
GA_1	8	7	8	10

[1]0.03 MM scale of the Aminco-Bowman spectrophotofluorometer. Excitation maximum 412-415 mu, emission maximum 465-470 mu.

Modifications of the Quantitative Method

Selection of the most suitable thin-layer plate.--Table 2 presents results of the comparative test of the 3 types of thin-layer plates considered for separation of the gibberellins from interfering substances. The Eastman silica gel plate required less time for development, gave better definition of the gibberellin area of the chromatogram and facilitated elution of the gibberellins from the plate.

Other modifications.--Several additional modifications were made to improve sensitivity and reproducibility of the quantitative procedure. All dilution of samples was eliminated during processing. In some of the first samples, GA from marker spots on the chromatographic plates drifted horizontally from the marker strip into the center portion of the chromatogram. This drift was eliminated by removing a narrow strip of silica gel from the polyester backing of the plate along the line delineating the marker strip. Further, all glassware was treated to remove fluorescing impurities from their inner surfaces. Glassware was washed in a non-fluorescing detergent, rinsed with methylene chloride, then with hexane, followed by methanol and finally put in concentrated sulfuric acid for at least 2 hours before rinsing with distilled water.

The final quantitative method.--The final modified procedure was as follows. The sample was blended for 2 to 4 minutes with acetone and water with an Omni-mixer. The amounts of acetone and water added depended upon the size of the sample. The homogenate was filtered through a Whatman 1 paper. The pulp and paper were blended with acetone for 3 to 4 minutes in the original Omni-mixer container. The homogenate was then filtered through a Whatman 1 paper on the original filter funnel. The Omni-mixer container was rinsed twice with small amounts of acetone and this acetone passed through the pulp. The pulp retained on the

Table 2.--Comparison of solvent flow, GA movement and GA definition on florisil, avicel and Eastman silica gel thin-layer plates.

Type of plate	Time for solvent[1] flow (minutes)	Definition of GA after movement	R_f
Florisil w/prewash	300	Fair	0.55 - 0.6
Florisil w/o prewash	300	Poor	0.55 - 0.6
Avicel	140	Fair	0.55 - 0.6
Eastman silica gel	90	Good	0.55 - 0.6

[1]Solvent system was chloroform, acetic acid, methanol (75:24:3).

filter was then washed with additional acetone. The combined filtrates were placed in a Florence flask and the acetone removed under vacuum, leaving the gibberellins and associated impurities in aqueous solution. Then, the aqueous solution was quantitatively transferred and filtered through a glass wool pledget overlaid on a Whatman 1 paper.

The filtrate was carefully measured and a portion taken which represented a 1-gram sample. This aliquot was acidified to pH 2.5 with 25% sulfuric acid and extracted 3 times with 10 ml portions of ethyl acetate. The combined ethyl acetate extracts were partitioned 3 times with 10 ml portions of water buffered at pH 7.0. Then, the combined aqueous extracts were acidified to pH 2.5 with sulfuric acid and partitioned 3 times against 10 ml portions of ethyl acetate. The combined ethyl acetate extracts were transferred to a 50 ml flask and the sample concentrated to dryness under vacuum. The residue was then dissolved in 0.5 ml of ethyl acetate and the solution subjected to thin-layer chromatography.

Since chromatography was a critical step, a detailed description of the preparation of the thin-layer sheet follows. Both the sample and a marker solution of GA were applied to the same plate 2 cm from the base. To separate the sample, applied in a thin line at the origin, from the markers of GA, applied in spots at both edges of the chromatogram, a 0.2 cm-wide silica gel strip was scraped from the polyester backing 2 cm from the edges vertical to the solvent. Thus, a marker of GA was on both sides of the chromatogram.

The chromatogram was developed with chloroform, acetic acid, methanol (75:24:3) until the solvent front had traversed 15 to 16 cm. The solvent front was marked and the chromatograms dried for 5 minutes. The marker strips were removed, sprayed with 90% ethanolic sulfuric acid,

heated at 100° C until dry (2-3 minutes), inspected under ultraviolet light and the blue, fluorescent marker spots marked. These marker strips were used to locate the gibberellin zones.

A zone 4 cm wide, centered on the marker spots, was cut from the chromatogram. The silica gel from this zone was scraped into a fritted glass funnel, the polyester backing rinsed and the compounds on the silica gel eluted from it with chloroform-methanol (20:10). The resulting sample was placed in a large test tube and the solvent removed under vacuum, leaving the residue containing the gibberellins in the test tube. Then, two ml of water was added to the test tube, the tube cooled in an ice bath and 10 ml of cold 85% sulfuric acid added as quickly as possible while the contents were vigorously stirred. After remaining in the ice bath for an additional 10 minutes, the samples were removed and allowed to stand overnight at room temperature before the RFI was determined.

Variability of the quantitative method.--RFI of 4 identical samples were determined to estimate the variability of the modified quantitative procedure. The RFI of the samples was 47.5, 49.5, 47.0 and 46.5. The maximum deviation of any single value from the mean of the 4 determinations was 4%, indicating satisfactory reproductibility of RFI using this procedure.

Influence of sample size on RFI.--In this test, it was demonstrated that sample size could be increased from 1 to 3 grams without dampening of RFI due to higher amounts of interfering substances in the larger samples. Comparative RFI of 1-, 3-, and 10-gram sample portions of a single sample is shown in Fig. 2. No appreciable dampening of RFI resulted when 3-gram sample portions were used in place of 1-gram portions.

Reliability of the sample weighing procedure.--Three samples were weighed before and after freezing. Fresh and frozen weights of the 3

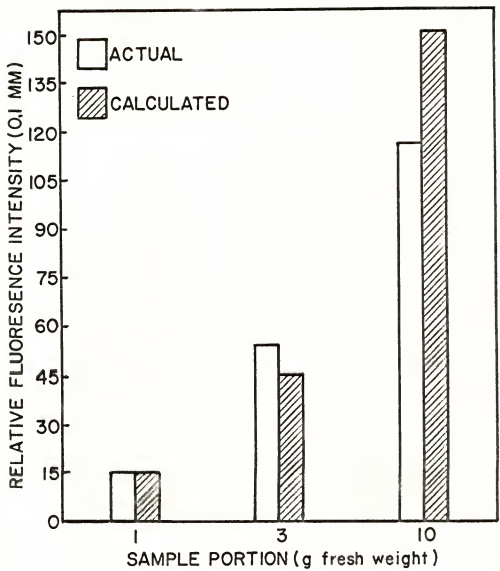

Fig. 2--RFI of processed 1-, 3-, and 10-gram portions of a single sample of navel orange fruits.

samples are shown in Table 3. Data from this experiment indicated that weighing of frozen samples was a satisfactory weighing method.

Variability and Sensitivity of the Fluorometric Procedure

Each of 5 different amounts of standard GA were added to 3 identical 1-gram fruit sample portions and RFI of the processed samples was determined. Samples in this triplicated test are shown in Fig. 3. The lowest concentration tested (2.66 mug per ml) did not give RFI which was significantly higher than the sample to which no GA had been added. Concentration differences from 5.2 mug per ml to 41.7 mug per ml were detected by the spectrophotofluorometer.

Verification of Gibberellin Activity in Processed Samples

Gas chromatographic characteristics.--A processed sample, standard GA and GA_1 and a solvent blank were propylated with diazopropane and the propylated solutions injected into a gas chromatograph.

Gas chromatographic spectra of the 4 solutions are shown in Figs. 4, 5, 6 and 7. Peaks appearing before 17 minutes after sample injection were due to solvents and solvent impurities. With 1 exception, all peaks occurring from 17 minutes to 48 minutes after sample injection were found in either the GA or GA_1 standards or in both.

Trapped portions of sample injections leaving the column from 17 to 48 minutes after injection had higher RFI than equal portions of the same sample that had not passed through the column (Table 4). The same was true of portions of the propylated GA_1 standard. These results indicated that the portion of the sample containing the characteristic peaks was also the portion responsible for the gibberellin fluorescence of the samples.

Barley endosperm bioassay.--Two samples processed to the point of final fluorescence development were assayed using the barley endosperm

Table 3.--Comparison of fresh weights and frozen weights of navel orange fruit samples.

Sample	Number of fruits	Fresh weight (grams)	Frozen weight (grams)	Error (grams)	Percent error
1	14	7.328	7.333	+.005	0.068
2	26	7.415	7.425	+.01	0.135
3	13	4.183	4.195	+.012	0.287

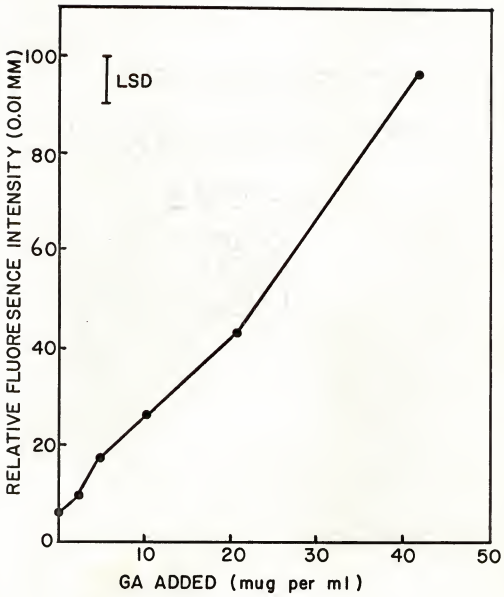

Fig. 3.--Mean RFI of processed samples to which GA was added. Each mean represents 3 determinations.

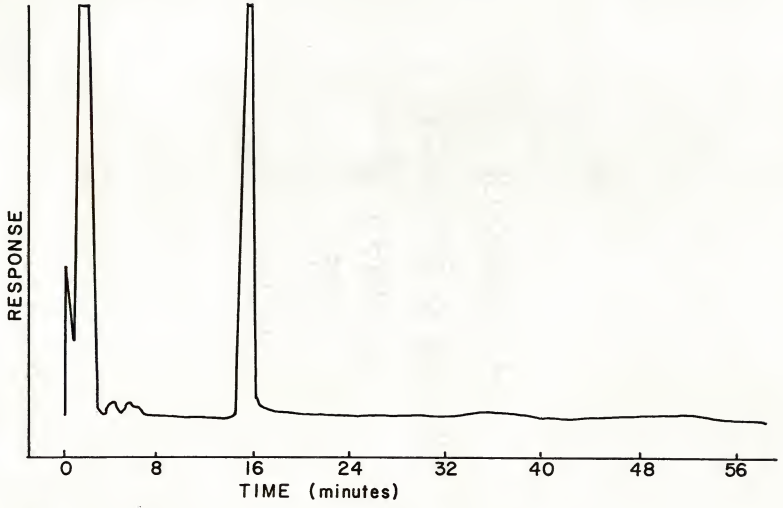

Fig. 4.--Gas chromatogram of the diazopropane blank.

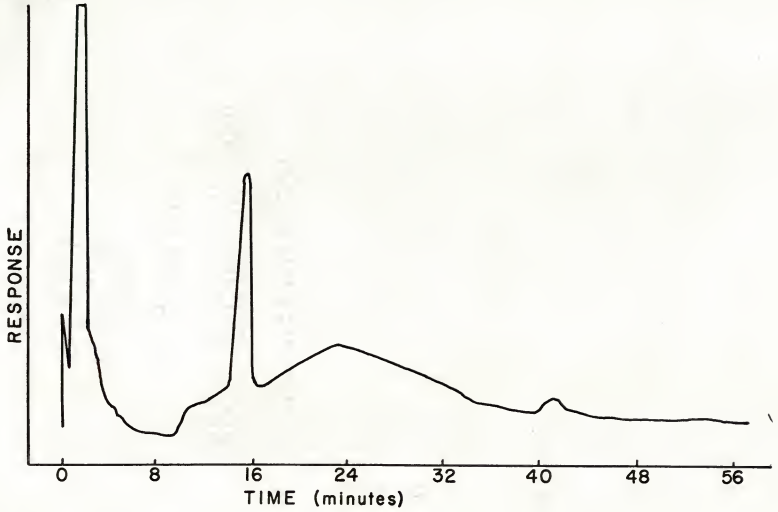

Fig. 5.--Gas chromatogram of propylated GA.

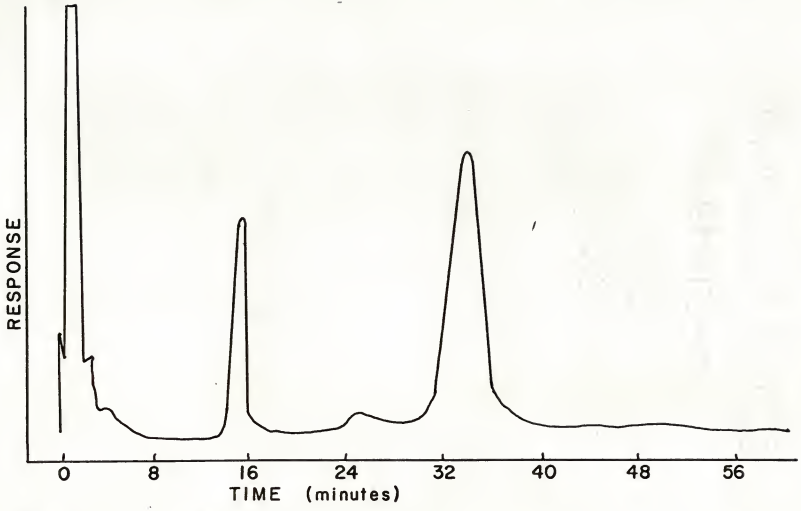

Fig. 6.--Gas chromatogram of propylated GA_1.

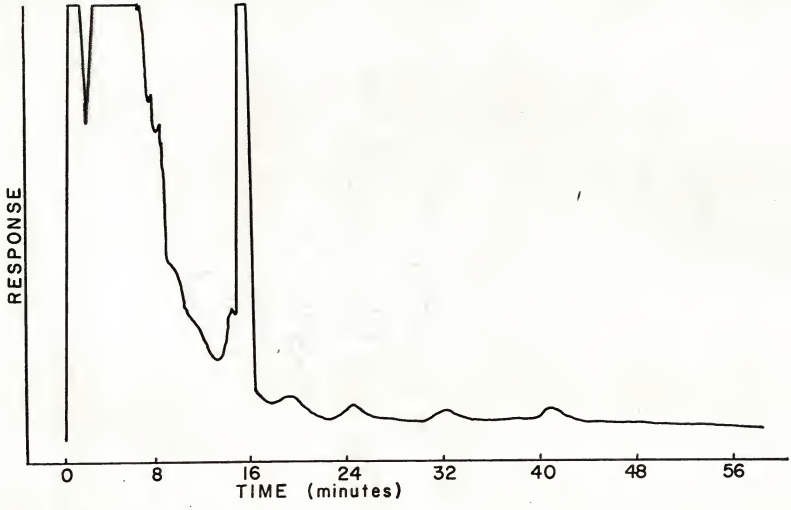

Fig. 7.--Gas chromatogram of a propylated fruit sample. Sample propylated after thin-layer chromatography but before treatment with sulfuric acid.

Table 4.--Comparison of RFI of sample and GA_1 solutions to RFI of propylated sample and GA_1 solutions separated by gas chromatography.

Solution	Relative fluorescence intensity[1]
Sample not propylated nor separated by GC	63
Sample propylated and separated by GC	108
GA_1 standard not propylated nor separated by GC	62
GA_1 standard propylated and separated by GC	109

[1]Measurements on 0.003 MM scale of the Aminco-Bowman spectrophotofluorometer, excitation maximum 420 mu, emission maximum 460 mu.

bioassay. Both samples tested gave a positive gibberellin response, indicating that gibberellins were present in the samples.

Fluorescence spectra.--Fluorescence excitation and emission maxima of processed samples agreed closely with published values. Discrepancy was apparently due to difference in spectrophotofluorometer calibration. Excitation and emission spectra of processed samples agreed with spectra of GA standards prepared in the laboratory and analyzed on the spectrophotofluorometer used for this research.

Studies of Endogenous Gibberellins in Navel Orange Fruits

Intertree Variability in Gibberellin Amounts

Samples were collected from 9 different trees and 1-gram and 3-gram sample portions were taken from each sample. The sample portions were then analyzed using the modified quantitative procedure. Results of the RFI determinations are shown in Table 5. Analysis of variance of these data showed a highly significant difference between RFI of 1- and 3-gram sample portions and a significant difference between mean RFI of the trees.

Sample Collection and Storage Methods and Intratree Variability in Gibberellin Amounts

Six samples were collected from a single tree and 3 different methods of sample handling were compared. In addition, 2 separate portions of each sample were analyzed in the laboratory. RFI of the samples analyzed in this experiment is shown in Table 6. Analysis of variance of these data showed that differences between collection and storage methods and differences between samples from the same tree were non-significant.

Seasonal Changes in Amounts of Endogenous Gibberellins

RFI of samples collected from the 4 experimental trees during the

Table 5.--RFI of processed sample portions from young fruit samples of 9 navel orange trees.

	Relative fluorescence intensity[1]		
	Sample portion size		
Tree	1 Gram	3 Grams	Mean
1	26.0	37.0	31.5
2	37.0	61.0	49.0
3	28.0	39.0	33.5
4	35.0	55.0	45.0
5	33.0	43.0	38.0
6	36.0	53.0	44.5
7	26.0	42.0	34.0
8	29.0	43.0	36.0
9	29.0	53.0	41.0
Mean	31.0	47.3	

[1]Measurements on 0.01 MM scale of the Aminco-Bowman spectrophotofluorometer, excitation maximum 420 mu, emission maximum 460 mu.

Table 6.--RFI of samples collected for comparison of sample collection and storage methods and for determination of intratree variability.

Treatment	Sample	Relative fluorescence intensity[1] Determination		Mean
Sample frozen in grove, stored for 3 weeks	1	38.0	37.5	
	2	45.0	36.0	39.13
Sample frozen in grove, analyzed immediately	1	38.0	38.0	
	2	44.0	39.0	39.75
Sample not frozen, analyzed immediately	1	43.5	43.5	
	2	45.5	42.0	43.63

[1]Measurements on 0.01 MM scale of the Aminco-Bowman spectrophotofluorometer, excitation maximum 417 mm, emission maximum 460 mu.

11-week sampling period was converted to GA equivalents per gram fresh weight of ovary tissue. GA equivalents were determined by comparing RFI of samples with GA standards and blanks. GA equivalents per sample were converted to concentration (mug GA per gram fresh fruit tissue) and total amount per fruit. Data from these determinations are shown in Table 7. Time of sampling is designated FB (full bloom), PF (petal fall) and the numbers following PF indicate weeks after petal fall. Petal fall was the time at which 2/3 of the petals had fallen from the axillary, leafy-bloom flowers on the tree. There was one missing value (PF plus 5 for tree 1) which was calculated using the method of Snedecor (242). The analysis of variance showed that highly significant differences existed between mean amounts of gibberellins in the ovaries and young fruits for the different sampling dates.

When mean concentrations (GA equivalents per gram fresh weight tissue) were compared using Duncan's multiple range test (65, 98), the results were as shown in Fig. 8. Gibberellin concentration was high at FB, dropped significantly at PF, rose significantly at PF plus 1 week and then remained significantly higher than the concentration at PF for the remainder of the sampling period. Concentrations during the period from PF plus 1 week to PF plus 6 weeks changed significantly, however.

Total amounts of gibberellin per fruit, calculated by multiplying mean concentration by the average fruit weight at the time of sampling, were as shown in Table 7 and Fig. 8. Comparisons of total amounts of gibberellins using Duncan's multiple range test (Fig. 8) showed that total amounts per fruit remained low during the first 4 weeks of sampling but increased significantly at PF plus 4 weeks and each week thereafter.

Growth of the Ovary and Young Fruit

Data for average weight and volume (calculated as the cube of fruit

Table 7.--Gibberellin concentration and total amounts of gibberellins per fruit in samples from 4 navel orange trees during the early fruit growth period, 1967.

Sampling date	Tree 1		Tree 2		Tree 3		Tree 4		Mean	
	Concentration[1]	Total per fruit[2]	Concentration	Total per fruit	Concentration	Total per fruit	Concentration	Total per fruit	Concentration	Total per fruit
FB (3/21/67)	126	5.34	181	10.28	131	6.49	182	9.10	155.0	7.80
FF (3/28/67)	82	8.77	50	9.49	73	14.49	38	6.27	60.8	9.76
FF+1 (4/5/67)	142	32.23	175	57.58	144	53.42	141	55.27	150.5	49.63
FF+2 (4/12/67)	142	54.53	113	62.94	128	77.31	139	86.74	130.5	70.38
FF+3 (4/19/67)	92	71.49	50	82.85	160	230.72	126	214.58	107.0	149.91
FF+4 (4/25/67)	157	203.32	138	638.43	128	448.26	130	367.25	138.3	414.32
FF+5 (5/2/67)	135	694.98	70	623.07	156	1078.74	132	942.48	119.3	834.82
FF+6 (5/9/67)	109	1156.28	101	1579.54	91	1367.55	87	1619.51	97.0	1430.72
FF+7 (5/16/67)	107	1804.02	107	2540.61	103	2475.91	142	3305.76	114.8	2531.58
FF+8 (5/23/67)	115	2783.00	107	3164.53	93	2939.45	94	3170.81	102.3	3014.45
FF+9 (5/30/67)	116	3885.19	103	3816.15	98	4266.23	107	4308.93	106.0	4069.13

[1]GA equivalents in mug per gram fresh weight fruit tissue.

[2]GA equivalents in mug per fruit.

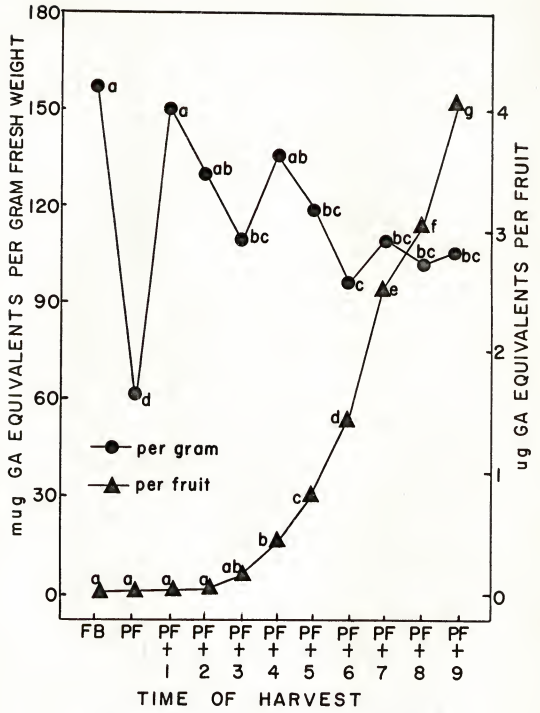

Fig. 8.--Seasonal changes in gibberellin concentration and total amounts per fruit of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance.

diameter) of the ovaries and young fruits and calculations of percent growth rate are presented in Tables 8 and 9. Fruit growth on the basis of either mean weight or mean volume followed the expected sigmoid growth curve of citrus fruits as described in previous research (10, 68, 109), and is plotted in Fig. 9. When mean weight and volume measurements were plotted semi-logarithmically (Fig. 10), 2 stages of fruit growth were apparent. From FB to PF plus 5 weeks,fruit growth was rapid, but variable and increased exponentially. After PF plus 6 weeks, fruit growth declined. Fruit growth up to the size of these samples at PF plus 5 weeks was considered to be due primarily to cell division (stage I of fruit growth) (10, 68, 137). Statistical analysis and comparison of mean weight and volume data for the different sampling dates using Duncan's multiple range test showed that the first significant increases in weight and volume occurred at PF plus 5 weeks, and that both weight and volume increased significantly each week thereafter. The results of the Duncan's multiple range test are shown on Fig. 9.

Weekly percent weight and volume increases (Tables 8 and 9 and Fig. 11) were used as indicators of weekly growth rate. These growth rates were determined by calculating weekly weight or volume increment as a percent of weight or volume at the beginning of the week. Statistical analysis of the percent growth rate data and comparison of the data using Duncan's multiple range test (Fig. 11) showed that significant changes in growth rate occurred during early fruit growth.

Relationships Between Gibberellins and Young Fruit Growth

Significant changes occurred in the amount and concentration of gibberellins in the ovaries and fruits of the navel orange during the period from FB until 9 weeks after petal fall. This was shown in Table 7, and Fig. 8. These significant changes in gibberellins were studied in relation to fruit growth.

Table 8.--Average fresh weight and percent weight growth of ovaries and young fruits in samples collected for determinations of endogenous gibberellins during growth of the young fruits.

	Averages per fruit									
	Tree 1		Tree 2		Tree 3		Tree 4		Mean	
Date of Sampling	Fresh weight (grams)	Growth rate[1] (%)	Fresh weight (grams)	Growth rate (%)	Fresh weight (grams)	Growth rate (%)	Fresh weight (grams)	Growth rate (%)	Fresh weight (grams)	Growth rate (%)
FB (3/21/67)	0.0424		0.0568		0.0496		0.05		0.0498	
PF (3/28/67)	0.107	152	0.1897	234	0.1855	274	0.165	230	0.162	225
PF+1 (4/5/67)	0.227	112	0.329	73	0.371	100	0.392	138	0.329	104
PF+2 (4/12/67)	0.384	69	0.557	69	0.604	63	0.624	59	0.542	65
PF+3 (4/19/67)	0.783	104	1.657	197	1.442	139	1.703	173	1.396	157
PF+4 (4/25/67)	1.295	65	3.153	90	3.502	143	2.825	66	2.694	93
PF+5 (5/2/67)	5.148	298	8.901	182	6.915	97	7.140	153	7.026	160
PF+6 (5/9/67)	10.608	106	15.639	76	15.028	117	18.615	161	14.973	112
PF+7 (5/16/67)	16.860	59	22.058	41	24.038	60	23.280	25	21.559	44
PF+8 (5/23/67)	24.200	44	29.575	34	31.607	31	33.732	45	29.779	38
PF+9 (5/30/67)	33.493	38	37.050	25	43.533	38	39.900	18	38.494	29

[1] Percent Growth Rate = $\frac{\text{Change in Average Fruit Weight During Week}}{\text{Average Fruit Weight at Beginning of Week}} \times 100$

Table 9.--Average volume and percent volume growth of ovaries and young fruits in samples collected for determinations of endogenous gibberellins during growth of the young fruit.

	Averages per fruit									
	Tree 1		Tree 2		Tree 3		Tree 4		Mean	
Date of Sampling	Volume[1] (cc)	Growth rate[2] (%)	Volume (cc)	Growth rate (%)	Volume (cc)	Growth rate (%)	Volume (cc)	Growth rate (%)	Volume (cc)	Growth rate (%)
FB (3/21/67)	0.0379		0.0486		0.0455		0.0455		0.045	
PF (3/28/67)	0.0954	152	0.2054	323	0.2106	363	0.1931	324	0.176	291
PF+1 (4/5/67)	0.2585	172	0.3779	84	0.439	108	0.480	149	0.389	121
PF+2 (4/12/67)	0.4987	93	0.7145	89	0.736	68	0.802	67	0.688	77
PF+3 (4/19/67)	1.0364	108	2.3953	235	1.996	171	2.217	177	1.911	178
PF+4 (4/25/67)	1.8974	83	4.683	95	5.169	159	4.189	89	3.985	109
PF+5 (5/2/67)	7.657	304	14.491	209	10.926	111	10.838	159	10.978	175
PF+6 (5/9/67)	17.014	122	26.331	82	24.642	126	30.635	183	24.656	125
PF+7 (5/16/67	29.532	74	37.193	41	43.726	77	38.375	25	37.207	51
PF+8 (5/23/67)	41.602	41	51.272	37	58.276	33	57.513	50	52.166	40
PF+9 (5/30/67)	56.623	36	60.698	18	76.766	32	69.089	20	65.794	26

[1]Calculated as cube of fruit diameter.

[2]Percent Growth Rate = $\frac{\text{Change in Average Fruit Volume During Week}}{\text{Average Fruit Volume at Beginning of Week}} \times 100$

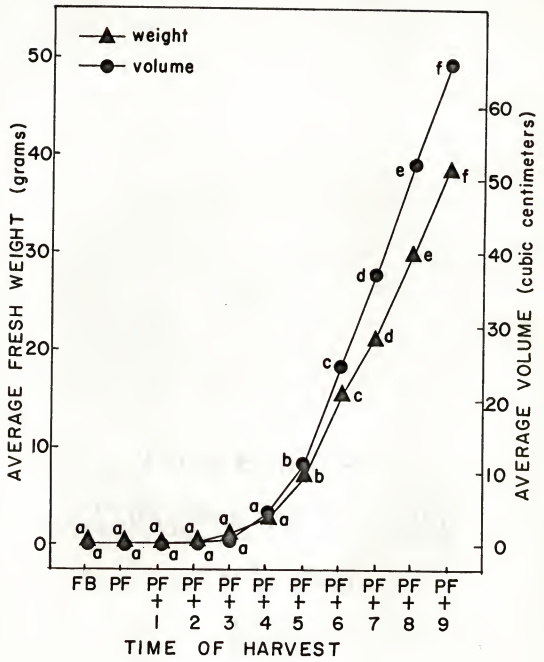

Fig. 9.--Seasonal changes in average weight and volume of fruits of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance.

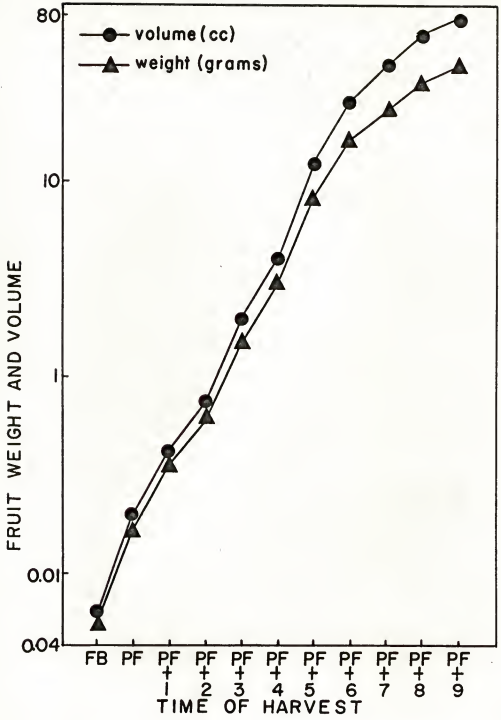

Fig. 10.--Seasonal changes in average weight and volume of fruits of samples of ovaries and young fruits of navel orange.

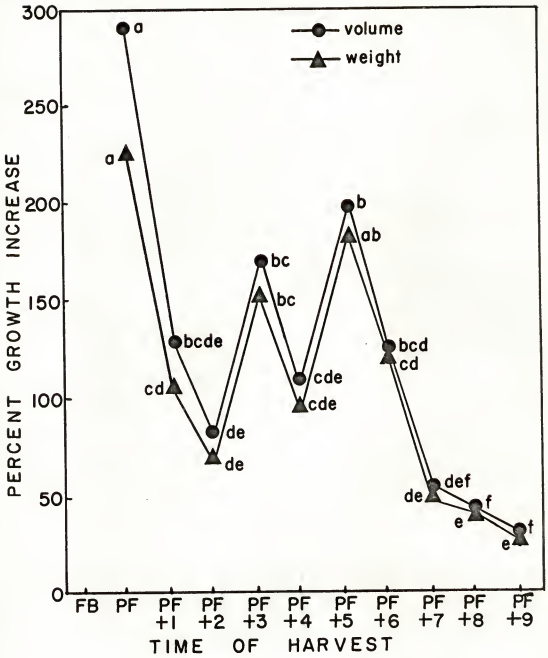

Fig. 11.--Mean weekly percent growth rate of fruits of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance.

Percent growth rate of the fruits (Tables 8 and 9 and Fig. 11) increased and decreased in a manner very similar to the increases and decreases in gibberellin concentration. Mean concentrations (mug GA equivalents per gram fresh weight fruit tissue) and percent weight growth for the 11 sampling dates are plotted in Fig. 12. Mean concentrations and percent volume growth for the 11 sampling dates are shown in Fig. 13. In both comparisons, significant changes in gibberellin concentration preceded similar significant changes in percent growth rate.

Referring specifically to the relationship between mean GA concentrations and percent weight growth data as shown in Fig. 12, a decrease in gibberellin concentration between FB and PF was followed by a decrease in percent weight growth rate between PF plus 1 week and PF plus 2 weeks. An increase in gibberellin concentration between PF and PF plus 1 week was followed by an increase in percent weight growth rate between PF plus 2 weeks and PF plus 3 weeks. A decrease in gibberellin concentration between PF plus 1 week and PF plus 3 weeks was followed by a decrease in percent weight growth rate between PF plus 3 weeks and PF plus 4 weeks. The same relationship continued until approximately PF plus 6 weeks, at which time the effect of gibberellin concentration on weight growth response of the fruit appeared to change.

In the first 3 weeks of young fruit growth there was a 2-week delay interval between change in gibberellin concentration and the percent weight growth response. The delay interval decreased to 1 week in the later part of the fruit growth period which is under study, due possibly to conditions more favorable for rapid growth response. When the time delay between changes in gibberellin concentration and changes in percent weight growth was taken into account, and pairings were made of the weekly change (+ or -) in gibberellin concentration and the weekly

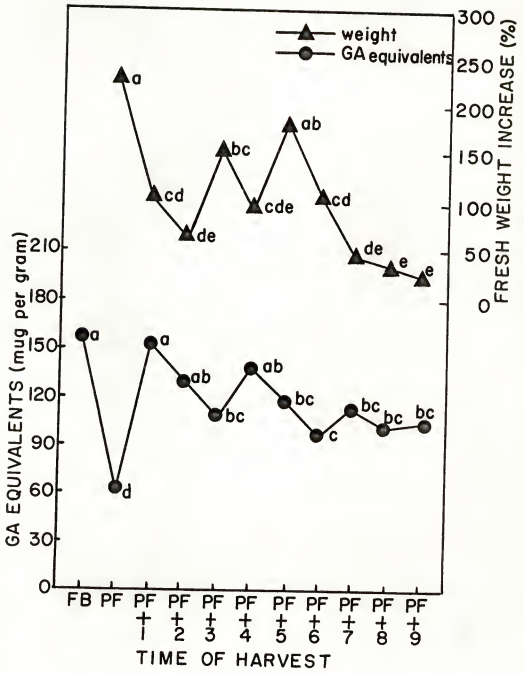

Fig. 12.--Mean weekly percent growth in fruit weight and gibberellin concentration of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance.

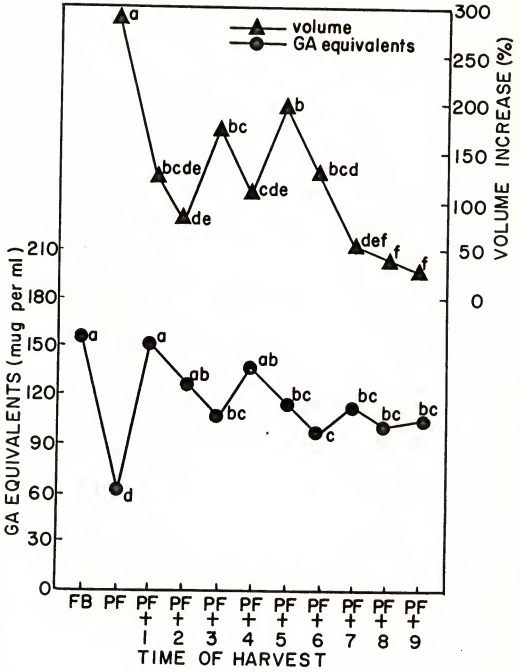

Fig. 13.--Mean percent growth in fruit volume and gibberellin concentration of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance.

change in percent growth rate during stage I of fruit growth, the results were as shown in the scatter diagram of Fig. 14. These paired values were taken from the individual data for the 4 experimental trees during the period from FB to PF plus 6 weeks. Correlation analysis of the 24 pairs of values gave a correlation coefficient of 0.703. Using Fisher's t test for correlation (242), this coefficient was significant at the 0.1% level.

A similar comparison was made of gibberellin concentration and percent volume growth and an almost identical relationship existed between gibberellin concentration changes and changes in percent volume growth, as shown in Fig. 13.

The effect of changes in total gibberellins per fruit on the cell enlargement phase of fruit growth is illustrated in Fig. 15 and Fig. 16. The first significant increase in total gibberellins occurred at PF plus 4 weeks while the first significant increase in mean sample weight occurred at PF plus 5 weeks (Fig. 15). Mean sample volumes and total gibberellins show an identical relationship (Fig. 16). Thus, at a time when young fruit growth due primarily to cell enlargement (stage II) is being initiated, total gibberellins per fruit increased significantly 1 week before the first significant increase in fruit weight or volume.

Estimation of the Major Fruit Drop Period

Table 10 presents data from the 2 counting methods which were used to estimate the major young fruit drop period. Weekly drop of ovaries and young fruit was high at FB and remained high until PF plus 4 weeks.

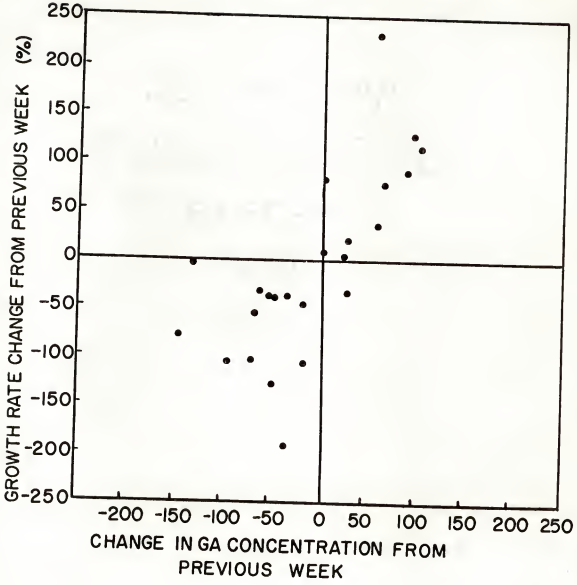

Fig. 14.--Scattergram of paired percent fruit weight growth and gibberellin concentration data of samples of ovaries and young fruits of navel orange.

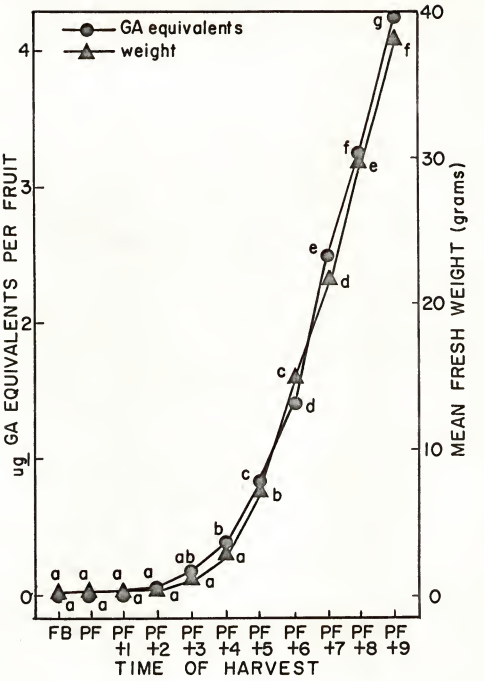

Fig. 15.--Seasonal changes in total gibberellins per fruit and mean weight of fruit of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance.

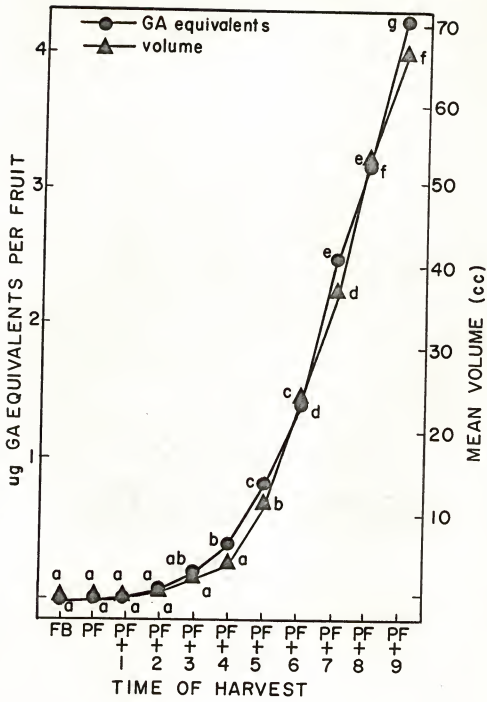

Fig. 16.--Seasonal changes in total gibberellins per fruit and mean volume of fruit of samples of ovaries and young fruits of navel orange. Letters in common indicate no significant difference in Duncan's Multiple Range Test at the 5% level of significance.

Table 10.--Young fruit drop from the 4 navel orange trees used in the experiment for determining changes in endogenous gibberellin amounts.

Stage[1]	Date	Fruit drop	
		No. fruit[2]	Percent[3,4]
FB	March 21	3667	
PF	March 28	2787	9
PF + 1 wk.	April 5	4190	38
PF + 2	April 12	6137	60
PF + 3	April 19	3914	72
PF + 4	April 25	785	20
PF + 5	May 2	251	8
PF + 6	May 9	355	18
PF + 7	May 16	143	11
PF + 8	May 23	152	12
PF + 9	May 30	64	4

[1]FB, full bloom; PF, petal fall.

[2]Counts of weekly drop onto screens placed beneath the tree.

[3]Percent Fruit Drop = $\frac{\text{Number of Fruit Abscissed During Week}}{\text{Number of Fruit at Beginning of Week}} \times 100$

[4]Branch counts included both bouquet and leafy bloom.

DISCUSSION

Analytical Methods

A major objective of this research was to find a method of quantitative gibberellin assay which was sufficiently sensitive for use with citrus ovaries and young fruits but which was simple enough that replicated experiments covering an extended period of time might be used. The complexity of the analytical method has seriously hampered research of this type in the past.

Based on the review of literature, the fluorometric method was selected as the least complex of available gibberellin assays. This method was investigated to determine its suitability for determining endogenous levels of gibberellins in ovaries and young fruit of the navel orange.

Although not the most sensitive assay method for gibberellins, the fluorometric method is more sensitive than most available bioassay methods. The Aminco-Bowman spectrophotofluorometer was sensitive to concentrations as low as 0.00488 ug per ml (1.45×10^{-8} M). Of the currently-available gibberellin assay methods, only the barley endosperm bioassay (189, 192) has been shown to be more sensitive. The barley endosperm bioassay has a lower sensitivity limit of 10^{-5} ug per ml (3×10^{-11} M). The 2 leaf disc bioassays which have been recently developed are reported to have greater sensitivity than the fluorometric method. The dandelion leaf disc bioassay (70) has a sensitivity range from 0.001 ug per ml to 10 ug per ml. The broad-leafed dock leaf disc

bioassay (292) is sensitive to gibberellin concentrations ranging from 0.00001 ug per ml to 0.1 ug per ml. The barley endosperm and the 2 leaf disc bioassays, while being more sensitive to gibberellins than the fluorometric method, require appreciable sample preparation before the bioassay can be conducted and the bioassay itself requires from 1 to 5 days.

Since the fluorometric method appeared less complex than other available methods and had been shown to have good gibberellin sensitivity, other parameters of the method were investigated.

Standard GA was added to plant extracts in order to determine the effect of interfering substances and to establish the variability and reliability of this method. That sensitivity of the Aminco-Bowman spectrophotofluorometer to low concentrations of gibberellins was not affected by interfering substances from citrus ovary and young fruit tissue was shown by the results of the triplicated variability and sensitivity test (Refer to Fig. 3). Variability between determinations was not excessive, as shown by the low LSD, and minimum sensitivity of the spectrophotofluorometer was 0.005 ug per ml.

The method of Gordon and Pankratz (85) was developed for determining GA alone and it has been shown in this research that GA_1, the only gibberellin definitely isolated from citrus tissue (257), can also be detected by the fluorometric method. Both GA and GA_1 are separated from plant tissue extracts by partitioning from acidic aqueous solution into ethyl acetate (67, 188), move in an identical manner on silica gel thin-layer sheets as shown in this and other research (67, 122, 124), fluoresce when treated with sulfuric acid and have identical fluorescence excitation and emission spectra. Thus, any GA applied to the plant for promoting fruit set, or GA_1 naturally present in the tissue, would be detected.

Since ovaries of navel orange flowers at anthesis weigh approximately 0.05 grams (20 ovaries per gram fresh weight), a quantitative method suitable for small samples was important. If excessively large numbers of ovaries must be collected in order to make laboratory determinations, the size of any planned experiment, or the number of replications per experiment, must be reduced accordingly. It has been demonstrated in this research that the gibberellins in samples as small as 1 gram fresh weight of ovary or young fruit tissue can be quantitatively assayed with reliability.

Although several changes were made in the procedure, these results were in agreement with results of other research performed to verify the occurrence of gibberellin-like substances in acidic-ethyl acetate-soluble fractions of plant tissue (12, 263). The gibberellin-like character of substances in the processed samples was verified using comparative gas chromatography, the barley endosperm bioassay and comparative fluorescence spectra.

Relative fluorescence intensity was found to be the most satisfactory method for quantitating GA and GA_1 in citrus fruit tissue. This conclusion was based on comparison of the complete fluorometric procedure with other methods which might have been used.

In selecting a quantitative method for gibberellin-like materials occurring in extremely low concentration in plant tissues, 2 important factors must be considered. The first is the actual quantitative measurement per se - its sensitivity, degree of complexity, availability of equipment and other considerations. The second factor is the amount of sample purification which must be done before the sample can be quantitatively assayed.

The Aminco-Bowman spectrophotofluorometer which was used in this

research can be used to detect very low concentrations of gibberellins. Development of sample fluorescence and the RFI determinations are relatively rapid. Quantitative bioassay procedures, on the other hand, require considerably greater expenditures of time, laboratory space and equipment in order to perform the assay. The barley endosperm bioassay (189, 192) is the least complex of the bioassay procedures but even this procedure requires a 24-hour incubation period for the endosperm pieces followed by laboratory analysis for reducing sugars. Thus, in the fluorometric method used in this research a relatively simple final physico-chemical assay was used rather than a bioassay.

Sample purification for gibberellin assay is generally very complex and the scope of gibberellin research is often reduced by the complexity of the extraction and purification procedure. Improper sample preparation can seriously affect the results of gibberellin assays. Weaver and Pool (283) and Coombe (47) reached different conclusions regarding amounts of gibberellins in grapes and this was attributed by Weaver and Pool to differences in sample purification method. The need for relatively pure samples before quantitation is common to all gibberellin assay methods, both bioassay and physico-chemical. A bioassay or physico-chemical method in which crude extracts could be assayed would be a definite improvement. It would also be of value if less complex methods for isolating gibberellins from sample contaminants could be developed. These could be goals of future research in gibberellin assay methods.

Studies of Endogenous Gibberellins in Navel Orange Fruits

In order to evaluate the data secured from the laboratory determinations of gibberellins in ovaries and young fruits of navel orange, it was necessary to determine the variability in amount of gibberellins be-

tween fruits of the same apparent physiological age on a single tree and the variability between fruits collected from different trees. Estimates of both intertree and intratree variability were secured prior to evaluation of the data from the major experiment for determining changes in endogenous gibberellin amounts during the young fruit growth period. Further, the experiments for determining intertree and intratree variability were designed to yield information on the effect of sample size on the final RFI determinations and the effect of collection and handling methods on the gibberellins contained in the fruit sample. These data were considered necessary for establishing the validity of the results of the major experiment and also to aid in design and execution of future experiments.

Intertree Variability in Gibberellin Amounts

In this experiment, comparison of the amount of gibberellins in young fruits from 9 different trees was secured. In addition, comparative RFI of 1- and 3-gram sample portions was determined.

When the data from this experiment (refer to Table 5) were subjected to analysis of variance, significant differences between mean amounts of gibberellins in samples from different trees were found. These significant differences indicated that replication was necessary in experiments of this nature.

Highly significant differences between RFI of 1- and 3-gram sample portions were also found in the analysis of variance of the data from this experiment. This was additional verification of the ability of the analytical method to detect differences in amount of gibberellins in fruit tissue samples.

Additional information was also secured from these data. The error mean square in the analysis of variance provided a means for determining

the precision of the analytical method. The standard deviation of a single RFI determination was calculated from the error mean square and found to be 3.72 points on the 0.01 scale of the spectrophotofluorometer. For 8 d.f. and 95% confidence, the least significant difference between any 2 single determinations was 12.13 points on the 0.01 MM scale of the spectrophotofluorometer. This information was valuable in the early stages of this research since it indicated that significant differences in amounts of gibberellins in navel fruit samples could be detected.

Sample Collection and Storage Methods and Intratree Variability in Gibberellin Amounts

In this experiment, comparative RFI of samples taken from the same tree, comparative RFI of identical samples collected and stored in different ways, and an estimate of the variability of the sampling and laboratory processing procedures were secured. When the data from this experiment (refer to Table 6) were subjected to statistical analysis, no significant differences were found between treatments (storage and handling methods) nor between samples from the same tree.

Since no significant differences were found between treatments, modifications can be made in methods of sample collection and storage in future research of this type. There was no significant deterioration of gibberellins when the samples were not frozen in the grove nor did the amounts of gibberellins in the samples decrease during the 3-week storage period. These results indicate that samples could be collected in the grove, frozen upon arrival in the laboratory and processed as time permits without concern for possible losses of gibberellins during sample storage. Such a system eliminates immediate freezing of samples in the grove which was very cumbersome.

The lack of significance between samples demonstrated the validity

of the main experiment for determining endogenous gibberellin levels during the early fruit growth period. In the main experiment, a single sample was collected and processed from each tree on each sampling date. The results of this experiment for determining intratree variability between samples indicate that a single composite sample gives an accurate estimate of gibberellin amounts of all fruit on the tree.

The lack of significance between samples found in the analysis of variance of these data also indicates that both sampling from the trees in the grove and the processing of the samples in the laboratory were done in a manner which did not induce error.

The analysis of variance of the data from this experiment provided additional information on the precision of the analytical method. The standard deviation of a single RFI determination was 3.14 points on the 0.01 MM scale of the spectrophotofluorometer. For 6 d.f. and 95% confidence, the LSD between any 2 single determinations was 12.13 points on the 0.01 MM scale of the spectrophotofluorometer. This LSD was almost equal to the LSD between any 2 determinations which was found in the intratree variability experiment, indicating again that the degree of precision which could be expected in the main experiment was sufficient for detecting differences in endogenous levels of gibberellin-like substances in the ovaries and young fruits.

Seasonal Changes in Endogenous Amounts of Gibberellins in Ovaries and Young Fruits

The major young fruit drop period occurred from full bloom until 4 weeks after petal fall. Both bouquet and leafy bloom fruit drop was included in the fruit drop estimates. Due to the complexity of the quantitative procedure, endogenous amounts of gibberellins were determined only for leafy-bloom fruits. Thus, direct comparisons of endogenous gibberellins in the samples and fruit drop can not be made. It

was made apparent by these data, however, that the sampling period included the period of young fruit drop.

Indirect evidence that gibberellins are controlling factors in fruit growth by regulation of both cell division and cell enlargement has been reported (14, 19, 24, 95, 141, 158, 219, 220, 279). Therefore, the significant changes in gibberellins which were found in this research were examined to determine any relationship existing between gibberellins and young fruit growth.

Two relationships between endogenous gibberellins and fruit growth were examined. These were the effect of changes in gibberellin concentration on stage I of fruit growth, the stage when fruit growth is primarily by cell divisions and,the effect of total gibberellins per fruit on the initiation of stage II of fruit growth, the stage when fruit growth is primarily by cell enlargement.

When percent growth rate of the fruits, on a basis of either fruit weight or fruit volume, was compared to gibberellin concentration, it was noted that fruit growth rate changed in a manner very similar to the changes in gibberellin concentration. Further comparison of fruit growth rate changes and gibberellin concentration changes indicated that significant changes in gibberellin concentration preceded similar changes in percent growth rate. This relationship continued until approximately petal fall plus 6 weeks, at which time the effect of gibberellin concentration on growth response appeared to change.

In the first 3 weeks of young fruit growth there was a 2-week delay interval between change in gibberellin concentration and the percent growth response. The delay interval decreased to 1 week in the later part of the fruit growth period which is under study, due possibly to conditions more favorable for rapid growth response. Taking the time

delay between change in gibberellin concentration and change in percent growth into account, pairings were made of weekly change in gibberellin concentration and weekly change in percent growth rate during stage I of fruit growth. Correlation analysis of these data showed that highly significant correlation existed between change in gibberellin concentration and change in percent growth rate. This highly significant correlation was considered very meaningful since gibberellin concentration was only 1 factor of the many which might affect young fruit growth.

An effect of changes in total gibberellins per fruit on fruit growth during stage II was also shown in the data from this research. The first significant increase in total gibberellins per fruit occurred at PF plus 4 weeks, while the first significant increases in mean sample weight and mean sample volumes occurred at PF plus 5 weeks.

These results indicated that at a time when young fruit growth due primarily to cell enlargement (stage II of fruit growth) was being initiated, total gibberellins per fruit increased significantly 1 week before the first significant increase in fruit growth.

A time-delay interval between stimulus and fruit drop has been described in citrus (44) and between gibberellin concentration change and growth response in other plants (182, 212, 213). The data from the experiment being reported suggests that a cause and effect relationship exists between gibberellins and growth of young navel orange fruit and that this relationship merits further investigation.

The significant changes in gibberellin concentration during bloom and the early period of fruit growth may be a possible cause for the variable results obtained when GA has been applied to navel oranges and tangelos for promotion of fruit set (35, 107, 108, 133). The response obtained may be affected by the endogenous concentration of gibberellins

in the fruit at the time of GA application. If GA were applied at a time when gibberellin concentration was at a critically low level, fruit set promotion could result. GA applied when endogenous gibberellin concentration was high might result in no increase in fruit set and possibly cause other undesirable responses such as leaf drop, fruit stem thickening or excessive splitting of the fruit.

SUMMARY

This research established that quantitative fluorometric measurement of gibberellins in ovaries and young fruits of the navel orange was feasible. The quantitative procedure, consisting of acetone-water extraction, solvent partitioning of the acidic, ethyl acetate-soluble fraction, thin-layer chromatography and relative fluorescence intensity measurement in 85% sulfuric acid, was used to measure gibberellins present in ovaries and young fruit during the bloom and early growth period of the fruits. Tissue concentration of gibberellins (GA equivalents per gram fresh weight fruit tissue) and total gibberellins per fruit were determined. Both gibberellin concentration and total amounts of gibberellins per fruit changed significantly during the 11-week period for which measurements were made.

Two relationships between young fruit growth and gibberellins in the fruits were examined. These were the effect of changes in gibberellin concentration on fruit growth during the stage of fruit growth when growth is primarily a result of cell division, and the effect of total amounts of gibberellins per fruit on initiation of the stage of fruit growth when growth by cell enlargement is predominant. In both of these relationships, there was a time delay interval between changes in gibberellins and the growth response. These data suggest a cause and effect relationship between gibberellins and fruit growth.

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BIOGRAPHICAL SKETCH

William Joseph Wiltbank was born January 1, 1927, in Clifton, Arizona and received his secondary education at Albuquerque High School, Albuquerque, New Mexico. After graduation from high school in 1944, he served in the U. S. Navy until his honorable discharge in June, 1946, with the rank of Electronics Technicians Mate First Class.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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